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COVID-19 prophylaxis with Doxycycline and Zinc in Health Care Workers: A prospective randomized double-blind clinical trial

Nejla Stambouli , Adel Driss , Faten Gargouri , Khadija Bahrini , Bilel Arfaoui , Rim Abid , Karima Taamallah , Souha Hannachi , Sana Boughariou , Aicha Rebai , Imen Naas , Mohamed Ghanem , Hassib Ammar , Chiraz Aichaouia , Asma Harrathi , Mohamed Ali Yousfi , Riadh Battikh , Mohamed Ben Moussa , Rabie Razgallah , Mustapha Ferjani , Hédi Gharsallah , on behalf of the of "OD-doxy-PNV-COVID-19 Trial"

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Res

- Using Doxycycline and Zinc as a potential treatment in SARS-COV-2 infection.
- SARS-CoV-2 contamination appeared to be reduced after treatment.
- COVID-19 infection risk was not associated with other comorbidities.
- The combined treatments of Doxycycline and Zinc have minimal side effects.

A prospective randomized double-blind clinical trial

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Ab

Background: SARS-CoV-2 is a novel virus that causes coronavirus disease-19 (COVID-19). Many antiviral and immunomodulatory drugs can be used as a potential treatment. Doxycycline combined to Zinc can play a pivotal role to protect against SARS-CoV-2 infection

Objective: This study aims to assess the efficacy of a combination treatment of Doxycycline and Zinc, in primary prevention of COVID-19 infection in Tunisian Health Care Workers (HCWs) compared to two control groups.

Methods: We conducted a prospective randomized double-blind clinical trial over five months to determine the efficacy of a combination preventive treatment dose of Doxycycline (100 mg/day) and Zinc (15 mg/day), compared to a single-dose treatment with Doxycycline versus a placebo. The effectiveness of preventive treatment was measured by the significant decline in the number of cases of COVID-19 infection and/or a decrease in the viral load determined by SARS-CoV-2 cycle threshold (Ct) value using RT-PCRs test.

Results: We detected a significant decrease of SARS-CoV-2 infection in group who received both Doxycycline and Zinc compared to other participants. We are also demonstrated that COVID-19 infection was not associated with diabetes (p=0.51), nor with hypertension (p=0.99), asthma (p=0.52) and chronic obstructive pulmonary disease (p=0.27).

Conclusions: Our finding indicated that preventive therapy reduced the risk of SARS-CoV-2. These results suggest that the combination of Doxycycline and Zinc has a protective effect in SARS-CoV-2 infection.

Keywords: Doxycycline; Zinc, Placebo, COVID-19Infection, Prophylactic.

A prospective randomized double-blind clinical trial

Introduction

Prevention of COVID-19 transmission and infection includes non-pharmacological intervention, and specific protection through chemoprophylaxis or immune-prophylaxis(Agrawal et al. 2020). Since the 1970s, the protective efficacy of Doxycycline has been demonstrated in the prophylaxis of malaria for travelers to endemic areas (Tan et al., 2011). In Tunisia, Doxycycline has been widely used in the prevention of malaria for military personnel going on missions to endemic areas(Ajili et al., 2013) . It has a low cost and a favorable safety profile, and has been proposed as a treatment for COVID-19 (Malek et al. 2020). Doxycycline has been used as a curative treatment for COVID-19 in Brazil and India(Christopher C Butler et al., 2021), while in the UK, it is recommended for suspected COVID-19 pneumonia in patients at high risk of adverse outcomes or where bacterial infection is suspected(Christopher C. Butler et al., 2021).

On the other hand, Zinc also has antiviral effects against certain viruses (Guo et al., 2004). In fact, Zinc supplementation improves the mucociliary clearance, strengthens the integrity of the epithelium, decreases viral replication, preserves antiviral immunity, attenuates the risk of hyper-inflammation, supports anti-oxidative effects, reduces lung damage and minimized secondary infections (Wessels et al., 2020).

The purpose of this study is to evaluate the efficacy and safety of Doxycycline with or without Zinc in preventing COVID-19 infection. The study focused on Tunisian health care workers (HCW) and the outcome measures were the decreased number of infected cases with COVID-19 and/or the decrease in the viral load, as determined by the cycle threshold (Ct values) of the RT-PCR tests for SARS-CoV-2.

Material and methods

1. Patients and methods

Weconducted a prospective randomized double-blind clinical trial during five months (from November 12,2020, through February 10, 2021). The study was conducted at different military site of investigation (Military hospital of Tunis, Military hospital of Bizerte, Military hospital of Gabes), was registered in the Clinical Trial database (NCT04584567) and approved by the local ethical committee of the General Directorate of Military Health.

or symptoms of respiratory infection (cough, fever>38.0°C, difficulty breathing, shortness of breath, chest pains) or other symptoms associated with COVID-19 like faintness, myalgia, headaches, and nausea or vomiting.

The study excluded HCWs with positive SARS-CoV-2 RT- PCRresults or positive SARS-CoV-2 serum tests (IgM or IgG). Positive SARS-CoV-2 RT-PCR wasdetermined by a cycle threshold (Ct) value below 33 using the Xpress SARS-CoV-2 kit (Xpert,Cepheid, Sunnyvale, CA, USA). The GeneXpert is a system that automates and integrates sample preparation, nucleic acid extraction, amplification, and detection of the target sequences using real-time RT-PCR assays. The real-time RT-PCR reagents, including primer N2 that target the virus nucleocapsid phosphoprotein (N) gene for specific detection of SARS-CoV-2 and human nucleic acid, respectively. The Cepheid Xpert Xpress SARS-CoV-2 kit detects N2 and E gene and solely N2 indicates positive results. The presence of only E indicates presumptive positive results while the presence of only SPC indicates negative results. The absence of all markers indicates an invalid result. (Moran et al., 2020),

Ct is a value that can broadly classify the concentration of viral genetic material as low, medium, or high in a patient sample using RT-PCR test. It tells us approximately how much viral genetic material is in the sample. A low Ct indicates a high concentration of viral genetic material, which is associated with high risk of infection. A high Ct indicates a low concentration of viral genetic material, which is associated with a lower risk of infection.

For the qualitative detection of antibodies against SARS-CoV-2, we used theBiosynex COVID-19 Ag+ BSS test (Illkirch-Graffenstaden, France).

In addition, we excluded HCWs who had a known hypersensitivity to Doxycycline or Zinc. HCWs with comorbidities (gastric bypass, epilepsy, cardiovascular disease, renal failure) or being treated with vitamin A during the study were excluded. Women's who were pregnant or nursing were also excluded.

Eligible participants were randomized in three parallel groups as follows:

Group A: Doxycycline (100 mg/ day) and Zinc (15mg/ day) combined orally for 6 weeks.

Group B: Doxycycline (100 mg /day) orally for 6 weeks.

Gro

Allocated study groups were randomly assigned by an interactive web-response system (Dacima), using simple 1:1 non-stratified sequence and a block size of 6. Allocation sequences are set automatically by the IWRS software.

Doxycycline was manufactured by Philadelphia Pharmaceutical (Batch number12927222). Placebo was also produced by the same manufacturer (Philadelphia Pharmaceutical). Placebo contains same excipients and no active substances. Zinc (Zinc Bisglycinate) was manufactured by Albion human nutrition (batch number F001).

The participants were screened 72 hours before enrollment. They were examined physically, tested for SARS-CoV-2 RT-PCR and serum test (IgM/IgG). All personal data was anonymized and signed Informed Consent Forms was obtained before the beginning of the study. The participants were monitored at days 21, 42 and 49 after enrollment. At each follow-up visit, participants were asked about their medical compliance and drug safety, underwent a clinical examination, and had an RT-PCR with calculated Ct values. After screening for patient's eligibility, a balanced randomization was done on the same day by the investigator. Allocated study drug was provided to the patient by the Military Hospital Pharmacist according to the allocation details, provided by the IWRS system. The patient received drug on the same day of the randomization. Study blindness was maintained by a blind allocation and drugs bottles were labeled anonymously by the pharmacist. Statistical analysis of the database was performed with blinded groups. Unblinding was performed at the final statistical report review. TheDacimaClinical Suite software (Montréal, Québec, Canada)was used to collect the data and to randomize participants based on FDA 21 CFR part 11 (Food and Drug Administration 21 Code of US Federal Regulations part 11), HIPAA (Health Insurance Portability and Accountability Act) & ICH (International Conference on Harmonization) requirements.

2. Study endpoints

The first endpoint of this study is to assess how Doxycycline with low dose can be used as a preventive treatment to decrease the number of cases infected with COVID-19 in the active arms compared to the placebo. Participants for each randomized treatment arm (Doxycycline only or combined with

Zin[illegible] of infected cases with COVID-19 and the viral RNA load after each treatment.

3. Sample size

Sample size was estimated by the Cochran-Armitage Test for Linear Trend in Proportions. Considering a sample power of 80%, an expected proportions of the primary endpoint of 8% in group A,10% in group B and 14% in group C, with a confidence level of 95%. The expected sample size was estimated up to 1,100 overall eligible subjects. However, due to the pandemic situation and the rush for nationwide vaccination against the SARS-CoV-2, the study was ended after including 172 randomized patients.

4. Evaluation:

During the follow-up period (6 weeks), we monitored the SARS-CoV-2 infection by RT-PCR tests at days 21 and 42 and 49. The last evaluation was done by day 49 to determine if a potential infection occurred after the treatment stopped at day 42.

5. Statistical analysis:

For statistical analysis, qualitative data was described by frequency and percentage of valid values. Continuous variables were used to describe mean and standard deviation (SD). Chi-square, Mann Whitney testand Kruskal-Wallis tests (one-Way ANOVA) were used to male inferential comparisons with a 5% significance level as a threshold. Differences were considered significant if $p \leq 0.05$. No further statistical analysis was performed due to study premature interruption.

Results:

From 193 selected HCWs 189 were screened. Four subjects declined to participate in the study. The screening phase excluded 17 subjects who were infected with COVID-19: positive SARS-CoV-2 RT-PCR (n=11); positive SARS-CoV-2 serology (n=6). Participants were not symptomatic at the time of RT-PCR/serology testing at inclusion (Figure1).

The remaining 172 eligible participants were randomized to one of the three study arms: 59 in the combined armDoxycycline and Zinc (group A), 56 in the Doxycycline only arm (group B) and 57 in the Placebo arm (group C).

38.0±10.6; 38.5±10.0 and 38.7±11.4 years respectively (p=0.934) (Table1).

The sex ratio was 1.6 (males =05, females =67), with no significant differences between the three studied groups (p=0.143). The study arms did not differ significantly for history of flu (n=43; 25.0%; p=0.927), hypertension (n=7; 4.1%; p=0.056), diabetes (n=4; 2.3%; p=0.162), asthma (n=2; 1.2%; p=0.590), and Chronic Obstructive Pulmonary Disease (COPD) (n=1; 0.6%; p=0.363) (Table 1).

All participants worked at the Military Hospital of Tunis, which has been active since the beginning of the COVID-19 pandemic. Among them, there were different levels of exposure to COVID-19 infection in the workplace, including invasive bedside procedures with COVID-19 positive patients (n=40; 23.3%), intra-operative procedure (n=19; 11.0%), and routine care (n=113; 65.7%), with no significant differences between the studied arms (p=0.2).

At the end of the study, a total of 24 subjects tested SARS-CoV-2 positive (14.0%). Among them, 5 (8.5%) in group A, 5 (8.9%) in group B, and 14 (24.6%) in group C, with a significant difference between the 3 arms (p=0.018) (Figure 2).

In the first group (group A), most participants (4/5) were asymptomatic, one participant developed symptoms with a persistent fever for 3 days. In the second group (group B) who have received only Doxycycline, two participants were asymptomatic and 3 developed moderate symptoms with fever, headache, and mild non-persistent cough. In the third group (group C) which received placebo, 6/14 participants developed symptoms such as fever, cough, and headache. For two of them the cough was persistent and corticosteroid treatment was prescribed. None of the participants in the three groups required oxygen (Table2).

This difference is also found from day 21 of the follow-up, when 9 subjects tested positive for SARS-CoV-2 RT-PCR (5.2%), as opposed to 2; 0 and 7 in the A, B and C groups respectively (p=0.01). Figure 2 shows the cumulative positive RT-PCR frequency trend among the three groups during the follow-up visits.

between the study arms revealed a significant difference in Ct levels($p<0.001$), with the highest mean level of Ct in group A (29.0±1.3) then groupB (22.8±4.0) then groupC (19.4±2.5). Table3 shows Ct profiles of the three groups during the study**.**

We observed that a higher risk of COVID-19 infection was not associated with diabetes ($p=0.51$), hypertension ($p=0.99$), asthma ($p=0.52$) or COPD ($p=0.27$). Table 4 shows the patient's baseline profile according to SARS-CoV-2 infection. Four of the 24 infected participants had comorbidities. In the combined treatment arm, there was no participants with comorbidities. In the single treatment arm, there was one diabetic and one hypertensive participant. In the placebo group, there was one participant with asthma and one participant with COPD. However, in the combined treatment arm there were no participants with comorbidities.

The safety of the Doxycycline/Zinc intervention was also assessed during the study follow-up. The most common adverse events reportedepigastralgia (n=14) and nausea (n=8). In term of side effects, the difference between studied groups were significant only for nausea ($p=0.032$), but not for epigastralgia ($p=0.249$)(Table5).

DISCUSSION

As of this date (January 2022), there are 106 clinical trials underway for prophylactic alternatives to COVID-19, some of which involve antivirals such as Favipiravir or interferons (Ben-Zuk et al. 2021). We remain the only study to evaluate the effect of Doxycycline in SARS-CoV-2 pandemic.

In addition to its anti-inflammatory effects proprieties. Doxycycline has also antiviral activity against several RNA viruses *in vitro*(Gendrot et al., 2020). It also chelatesZinc from metalloproteases (MMPs) (Sodhi and Etminan, 2020).As a result, their chelating activity may help to inhibit SARS-CoV-2 infection by limiting its ability to replicate in the host, thereby acting as a prophylactic agent (Sodhi and Etminan 2020) .

There have already been some recommendations of Doxycycline for COVID-19, particularly in patients with pneumonia and those who are at high risk of complications. There is now evidence of increased use of respiratory antibiotics, including Doxycycline, during the COVID-19 pandemic in both the UK and USA(de Lusignan et al., 2021.).

only Doxycycline or Placebo as prophylaxis for COVID-19 in Tunisian HCW.

In this trial, however, the results are under-powered (69.2%), as we were unable to meet the scheduled sample size estimated at a total of 1,100 samples. Moreover, the recruitment of HCWs remains difficult because the enrollment of patients overlapped with the vaccination campaign. The study recruited only 15.6% of the estimated sample size.

A prophylactic effect observed with Doxycycline may be attributed to the fact that it inhibits MMPs. In fact, it has been demonstrated that coronaviruses exploit MMPs for a range of activities (replication, cell infection and survival). Therefore, Doxycycline may have an antiviral effect on SARS-CoV-2 (Dutta and Basu, 2011).

Researchers have suggested that Doxycycline may delay COVID-19 progression via anti-inflammatory activities, including viruses that regulate the NF-ƙB pathway (nuclear factor kappa light chain enhancer of activated B cells) and inhibit of proinflammatory cytokine levels (IL-6, IL-1β, TNFα), during acute respiratory distress syndrome (ARDS) in severely ill COVID-19 patients(Anwar et al., 2020). Earlier studies demonstrated the effectiveness of chemically modified tetracyclines against SARS, preventing septic shock and ARDS development (Griffin et al., 2010).In addition, several case reports suggest beneficial effects of Doxycycline preventive treatment. In this context, a study have demonstrated an improvement of clinical symptoms after treatment with standard doses of Doxycycline in 4patients COVID-19 with a high-risk pulmonary disease(Yates et al., 2020).

In addition, the combination of Doxycycline and Zinc also seems interesting, since Doxycycline has been shown toprotectagainst lung infection by inhibiting MMPs, in which it is dependent on Zinc (Doroszko et al., 2010). An interesting result of this clinical trial is the reduction in viral load in the combined group therapy(Doxycycline and Zinc) as compared to other groups. This finding is supported by cumulative Ct valuesat day 49, which shows an inverse relationship with viral mRNA (Supplementary Figure 1). These results are in agreement with other studies, which have suggested that Ct values are inversely associated with the viral load, and every 3-fold increase in Ct values indicates a 10-fold decrease in starting material(Tom and Mina, 2020). Low Ct values were also reported to be associated with virus growth in cell

cult[illegible]Ps with Doxycycline may help repair the damaged lung tissue, thus decreasing the virus's availability in the nasal tract and enhancing recovery.

The potential effect of combined therapy may be explained by the ability of effect Doxycycline's ability to catalyze Zn2+ ions, which are required for the activity of MMPs, independently of its antimicrobial properties (Castro et al., 2011). Doxycycline is the most potent tetracycline derivatives inhibitor of MMPs, even at low doses (25 mg) (Castro et al., 2011). It may act as an ionophore by increasing intracellular Zinc concentrations, suppressing viral replication and strengthening the immune system (Griffin et al., 2010; te Velthuis et al., 2010).In addition to Doxycycline effect, Zinc have also a benefic role to protect against COVID-19 infection. In fact, many reports have shown that Zinc can inhibit the enzymatic activity and replication of SARS-CoV-2's RNA polymerase, and can inhibit angiotensin converting enzyme ACE2 activity (Skalny et al., 2020;Ratia et al., 2006). It wassuggestedthatZinc can prevent fusion with the host membrane, decreases the viral polymerasefunction, impairs protein translation and processing, blocks viral particle release, and destabilizes the viral envelope(Wessels et al., 2020). Therefore,Zink has anti-inflammatory and anti-oxidative properties and underlying mechanisms have been the focus of numerous studies(Wessels et al., 2017).

In this study, we recruited 193 participants among HCW for 4 months (from November 2020 to February 2021). The percentage of HCW who tested positive for COVID-19 was 21,24%. A meta-analyses conducted during the first 6 months of the pandemic from January 01to July 09, 2020, found a percentage of infected HCW of 51.7% (Fawad et al., 2021). These results show that HCW are at high risk of COVID-19 infection, and thus, prevention strategies should be developed in this category. This will have a positive impact on patient's safety and could decrease the absenteeism level.

Our study was stopped after 4 months, due to the start of the vaccination campaign, which prioritized HCW(Kefi et al., 2021). We achieved 15.6% of the estimated sample size. Therefore, we recommend additional research on chemoprophylaxis that could enhance COVID-19 vaccine and litigation strategies to protect against it.

Ochoa et al., 2021), they were not significantly associated with an increased risk of infection in the sample studied. Moreover, we did not consider the compliance of the HCW with the protective measures. The risk of COVID-19 infection directly depends on our physical prevention measures.

Despite the low number of participants in this study, the data suggest that a double treatment of Doxycycline and Zinc may have a potential preventive effect. Public health suggestions can be based on this information for a wider adoption of prophylaxis treatment in HCWs and the general population. Additional studies should be conducted considering vaccination laws.

Conclusion

The only indication approved by the US Food and Drug Administration (FDA) for the use of Doxycycline in patient is as a malaria prophylaxis (< 4 months). According to the portal clinicaltrials.gov, to date, there are not less than 405 clinical trials registered involving Doxycycline targeting different diseases such as bacterial infections, cystic fibrosis, acne, vascular diseases, and HIV-AIDS.However, the prescription of long-term antibiotics must be cautious and supervised. The overuse is linked to bacteria resistance and alterations in gut microbiota, which has been related to risks of various chronic diseases. In the present study, Doxycycline combined or not to Zinc has been used before the beginning of vaccination campaign. Nevertheless, this alternative therapy might be useful for certain HCW and/or others who have comorbidities and aren't responding to vaccines or have refused them.

Taking preventive measures decreased the risk of contamination by SARS-CoV-2. In this study, Doxycycline and Zinc were found to have a protective effect when taken together. Chemoprophylaxis can be used in conjunction with COVID-19 vaccination and protective measures, but more research is needed.

Abbreviations:HCW: Health care workers; DOXY: Doxycycline; ICF: Informed Consent Form; FDA 21 CFR part 11: Food and Drug Administration Code 21 of US Federal Regulations part 11; HIPAA: Health Insurance Portability and Accountability Act; ICH: International Conference on Harmonization; SD: Standard deviation; RT-PCR: Real time polymerase chain reaction; Ct: Cycle threshold; *COPD : Chronic*

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ARDS: Acute respiratory distress syndrome; MMPs: Matrix metalloproteinases.

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Ethical statement: This study has been approved by the local ethics committee of the General Directorate of Military Health.

Conflict of interest statement: The manuscript is an original work that has never been published or is under consideration for publication in another journal. We confirm that all authors have participated in the study and have approved the manuscript.

Contributions: NS, HG, RB, MBM, MAY: Protocol redaction; NS, RB, MBM, HG: Conceptualization; NS, RB, MBM, HG: Methodology; NS, KT, RB, HG: Formal analysis; NS, KB, AD, RB, GH, RR: Writing; SN, AD, BA, KB, RB, HG: Review and editing. AB, RA, SH, SB, AR, NI, MG, AH, CA, AH, FG: Investigation; MAY: Product manager, supervision; MBM: Virological testing, RR, KT: Validation, resources, software, visualization, statistical analysis; MF, HG: Project administration.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Fig ...ne three study arms: 59 in the combined Doxycycline and Zinc arm, 56 in the Doxycycline only arm, and 57 in the Placebo arm.

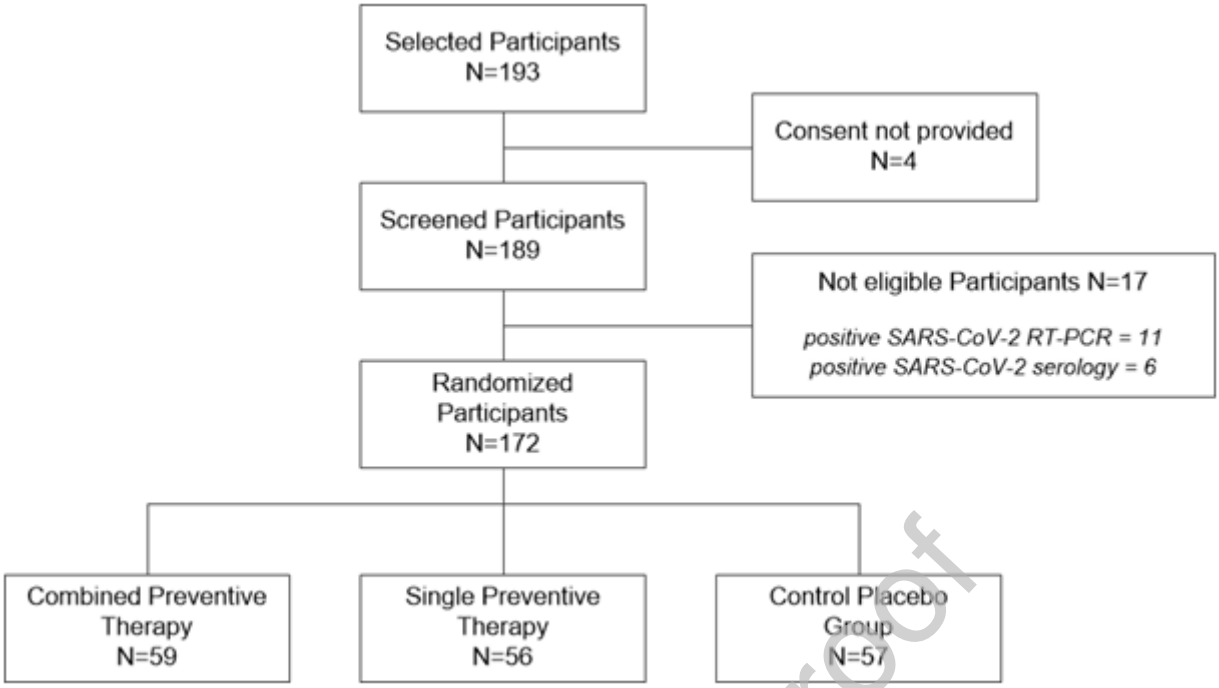
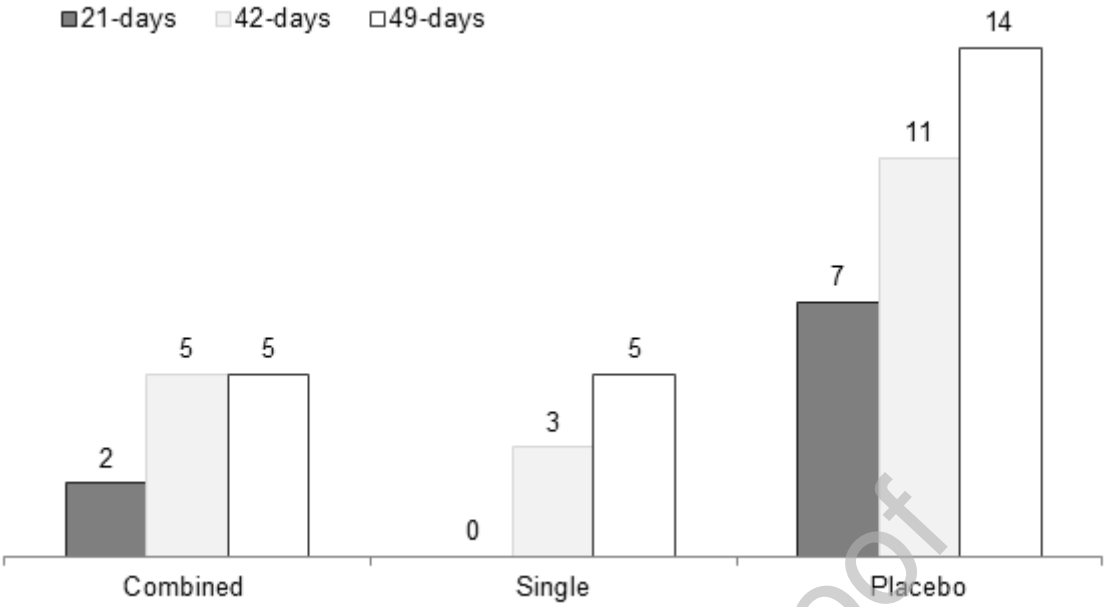


Fig [illegible] up (p=0.018). Each group's histogram represents the number of days during follow-up. 24 days are represented by black, 42 days by gray, and 49 days by white.



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	All participants N=172	Group A N=59	Group B N=56	Group C N=57	p value
Age	38.4±10.7	38.0±10.6	38.5±10.0	38.7±11.4	0.936
Gender ratio	1.6 (105M/67F)	2.5 (42M/17F)	1.2 (31M/25F)	1.3 (32M/25F)	0.143
Comorbidities (n, %)		14	17	19	
Hypertension	7 (4.1%)	0 (0%)	2 (3,6%)	5 (8,8%)	0.056
Diabetes	4 (2.3%)	0 (0%)	1 (1,8%)	3 (5,3%)	0.161
Asthma	2 (1.2%)	0 (0%)	1 (1,8%)	1 (1,8%)	0.589
COPD	1 (0.6%)	0 (0%)	0 (0%)	1 (1,8%)	0.363
History of Covid19 infection	0	0	0	0	_
Vaccination		Vaccine not yet available			

NS: Not Significant; COPD: Chronic Obstructive Pulmonary Disease

Table 2: Clinical symptoms of COVID-19 infected participant

Symptoms	Combined treatments (Group A)	Single treatment (Group B)	Placebo (Group C)	Total
Asymptomatic	**04**	**02**	**08**	**14**
Mild to Moderate	**01**	**03**	**06**	**10**
Total	**5**	**5**	**14**	**24**

Table 3: Total Cycle threshold profile of the three groups during the study.

Study Arm	Mean±SD	CI 95%	p value
Combined Therapy	29.0±1.3	[27.7; 30.3]	<0.001
Single Therapy	22.8±4.0	[18.6; 26.9]	
Control Group	19.4±2.5	[17.8; 21.0]	
Overall	22.6±4.7	[20.6; 24.7]	

SD: standard deviation; **CI:** confidence interval. Comparison between three groups using Kruskall Wallis analysis. Results were considered significant if p value <0.05

Table 4: Patient baseline profile according to SARS-CoV-2 infection

Endpoint	Infected patients with SARS-coV-2	Non-infected patients	p value
N	24	148	-
Diabetes	1 (4.2%)	3 (2.0%)	0.518
HTN	1 (4.2%)	6 (4.0%)	0.999
Asthma	1 (4.2%)	1 (0.68%)	0.520
COPD	1 (4.2%)	0 (0.0%)	0.279
Conveyance **Own car** **Public Transport**	 8 (33.34%) 16 (66.67%)	 60 (40.55%) 88(75.68%)	0.502

HTN: Hypertension; COPD:Chronic obstructive pulmonary disease

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Adverse Event	Combined Therapy	Single Therapy	Control group	Overall	p value
N	59	56	57	172	-
Epigastralgia	7 (11.9%)	5 (8.9%)	2 (3.5%)	14 (8.1%)	0.249
Nausea	1 (1.7%)	6 (10.7%)	1 (1.8%)	8 (4.7%)	0.032
Overall	8 (13.6%)	11 (19.6%)	3 (5.3%)	22 (12.8%)	0.071